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MINIREVIEW

# Role of *Toxoplasma gondii* IgG Avidity Testing in Discriminating between Acute and Chronic Toxoplasmosis in Pregnancy

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**ABSTRACT** Risk of mother-to-child transmission of *Toxoplasma gondii* during pregnancy is much greater in women who are exposed to primary *T. gondii* infection (toxoplasmosis) after conception compared to those who were exposed to the infection before conception. Therefore, laboratory tests that help classify recent primary toxoplasmosis are important tools for the management of pregnant women suspected to have *T. gondii* exposure. Detection of *Toxoplasma* IgM (Toxo IgM) is a sensitive indicator of primary toxoplasmosis, but the indicator specificity is low because sometimes natural IgM antibodies react with *Toxoplasma* antigens in the absence of the infection. Furthermore, Toxo IgM sometimes persists in blood serum for several months or years following the primary infection. In recent decades, Toxo IgG avidity assay has been used as a standard diagnostic technique for a better estimation of the infection acquisition time and identification of the primary *T. gondii* infection during pregnancy. Avidity is described as the aggregate strength; by which, a mixture of polyclonal IgG molecules reacts with multiple epitopes of the proteins. This parameter matures gradually within 6 months of the primary infection. A high Toxo IgG avidity index allows a recent infection (less than 4 months) to be excluded, whereas a low Toxo IgG avidity index indicates a probable recent infection with no exclusions of the older infections. This minireview is based on various aspects of *T. gondii* IgG avidity testing, including (i) description of avidity and basic methods used in primary studies on *T. gondii* IgG avidity and primary infections; (ii) importance of IgG avidity testing in pregnancy; (iii) result summary of the major studies on the use of *T. gondii* IgG avidity assay in pregnancy; (iv) brief explanation of the *T. gondii* IgG avidity values in newborns; (v) result summary of the major studies on *T. gondii* IgG avidity and PCR; (vi) discussion of commercially available *T. gondii* IgG avidity assays, including newer automated assays; and (vii) current issues and controversies in diagnosis of primary *T. gondii* infections in pregnancy.

**KEYWORDS** acute toxoplasmosis, chronic toxoplasmosis, congenital toxoplasmosis, IgG avidity, pregnant women, *Toxoplasma gondii*

*Toxoplasma gondii* infection (toxoplasmosis) is one of the most important parasitic protozoan infections in humans and warm-blooded animals worldwide (1). Sources of this parasitic infection include ingestion of raw and/or undercooked meats with the parasite tissue cysts, sporulation of oocysts from consumption of contaminated vegetables and water, as well as accidental ingestion of contaminated soil. Vertical transmission from pregnant women with primary infections to their fetuses may result in congenital toxoplasmosis (CT). In fact, CT occurs predominantly after primary maternal

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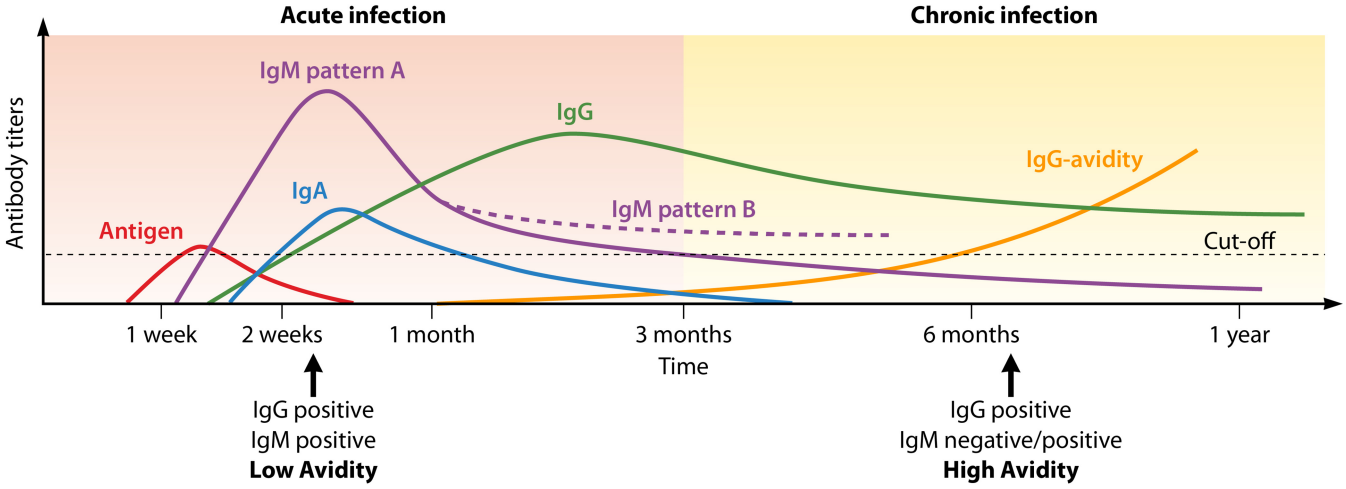


**FIG 1** Relative changes in Toxo IgM, IgG, and IgG avidity over time following primary infection. IgM pattern A represents the typical IgM response pattern, whereas IgM pattern B represents long-term IgM persistence.

*T. gondii* infection during or shortly before pregnancy (2). However, transmission of the parasite has been reported from recently infected women (immediately prior to pregnancy), immunosuppressed reactive women, and previously infected pregnant women who develop infections with novel serotypes (3). Although CT includes a broad range of clinical symptoms, the infection is subclinical in approximately 75% of the infected neonates. Severity of the clinical disease in congenitally infected infants is inversely correlated to the gestational age at which the primary maternal infection is acquired. Clinical manifestations of CT may result in severe damages to the fetus, including retinochoroiditis and serious developmental disorders such as hydrocephaly, microcephaly, and mental retardation. Moreover, spontaneous abortion, prematurity, and stillbirth may occur (4, 5).

Studies have shown strong associations between the primary *T. gondii* infections in mothers and *in utero T. gondii* transmissions. Risk of mother-to-child transmission (MTCT) of *T. gondii* in congenital infections varies with the trimester during which the maternal infection is acquired. Risk of MTCT in untreated women is approximately 10 to 15, 30, and 60% for acquisitions during the first, second, and third trimesters, respectively (6). In a meta-analysis of 22 European cohorts on women screened routinely during their pregnancy and treated accordingly once the primary infection was diagnosed, the MTCT rate was less than 5% when the acute primary maternal infection was detected very early in pregnancy. However, the MTCT rates were much higher in acute maternal infections acquired later in pregnancy, including 15, 44, and 71% after maternal seroconversions at 13, 26, and 37 weeks of gestation, respectively (7). In rare cases, congenital transmission occurs in chronically infected women, whose infections have been reactivated due to their immunocompromised conditions, such as AIDS and corticosteroid therapy (8–10).

Established links between the primary *Toxoplasma* infections in pregnancy and congenital infections urge identification of the primary *T. gondii* infection as an important goal in maternal and neonatal safeties. However, most pregnant women with acquired acute infections do not experience significant symptoms or signs and, hence, cannot be diagnosed on clinical grounds (11). Documentation of seroconversion during pregnancy is the most direct indicator of primary toxoplasmosis. However, due to the lack of preconception antibody screening programs that allow detection of seronegative women, this approach is rarely effective. Diagnosis of toxoplasmosis, which is often asymptomatic, is primarily based on serological tests that detect *T. gondii*-specific IgG and IgM antibodies. Usually, specific IgM appears nearly 1 week after the exposure (12) and IgG appears 1 to 3 weeks after IgM appearance (Fig. 1). Absence

of IgM usually shows evidence of past infections of *T. gondii*, while presence of the antibodies demonstrates acute infections (13). Therefore, most studies focus on detection of *T. gondii* IgM due to its well-known use as a transient indicator of primary infection. However, discrimination between past and recent infections is challenging because *Toxoplasma* IgM (Toxo IgM) can persist for several months or years following the primary infection (4, 6, 14–16). Furthermore, natural IgM antibodies sometimes react with *Toxoplasma* antigens in the absence of the infection (14, 17). These findings for *T. gondii* IgM have led to a search for different laboratory assays that can be used to identify primary *T. gondii* infections (6, 17). During the past few decades, Toxo IgG avidity assay has been used as a standard diagnostic technique for improved estimation of infection acquisition time worldwide and identification of primary *T. gondii* infections in pregnancy (18, 19). It has been shown that IgG avidity testing can provide confirmatory evidence of acute infections and discriminate between reactivations and primary infections using a single serum sample. This is especially important in pregnant and immunosuppressed patients (20–24). The present minireview includes various aspects of *T. gondii* IgG avidity testing, including (i) description of avidity and basic methods used in primary studies on *T. gondii* IgG avidity and primary infections; (ii) importance of IgG avidity testing in pregnancy; (iii) result summary of the major studies on the use of *T. gondii* IgG avidity assay in pregnancy; (iv) brief explanation of the *T. gondii* IgG avidity values in newborns; (v) result summary of the major studies on *T. gondii* IgG avidity and PCR; (vi) discussion of commercially available *T. gondii* IgG avidity assays, including newer automated assays; and (vii) current issues and controversies in diagnosis of primary *T. gondii* infections in pregnancy.

## DEFINITION OF AVIDITY AND BASIC METHODOLOGY

The IgG avidity test was first described by Hedman et al. in Finland (18). Avidity is described as the aggregate strength by which a mixture of polyclonal IgG molecules reacts with multiple epitopes of the proteins. Functional binding affinity of anti-*T. gondii* IgG increases progressively after immunities from infections and is otherwise referred to as maturation of the humoral immune responses. Low IgG avidity indices usually specify the first few months of primary infections, whereas high-avidity indices specify nonprimary infections (25). The basic methodology used to assess avidity is based on the weak binding of low-avidity IgG to a mixture of *T. gondii* antigens. Antigen-bound low-avidity IgG is easily broken from the antigen in the presence of mild protein denaturants, such as urea, potassium thiocyanate, and guanidine chloride, while high-avidity antibodies remain bound to the antigen (Fig. 2) (26). An enzyme-linked immunosorbent assay (ELISA) using urea as the dissociating agent is the most common test format (25, 26). The methodology is further detailed as follows. Patient diluted serum is added to two rows of a plate coated with *T. gondii* antigen. After incubation, one row of the plate is washed using regular wash buffer, whereas the other row is washed using wash buffer containing urea. Then, ELISA is terminated via routine procedures, and the optical density (OD) of each well is measured using an automated ELISA reader at 492 nm. Results are generally expressed as an avidity index (AI), calculated using the following formula (27): AI (%) = (OD of washed urea well/OD of washed regular buffer well) $\times$ 100.

## IMPORTANCE OF IgG AVIDITY TEST IN PREGNANCY

Assessment of *Toxoplasma*-specific antibody status in pregnant women during the first trimester of pregnancy is extremely important. Diagnosis of acute toxoplasmosis helps prevention of congenital infections in fetuses and provides opportunities to carry out early therapies or other interventions (28–31). The IgG avidity usually shifts from low to high within 5 to 6 months of the primary infections. It is particularly effective in pregnant women, whose tests for IgG and IgM against *Toxoplasma* are positive during their first months of gestation (32). For example, women with high-avidity tests in their first trimester do not show acute infections in the last 3 months. This can be used to rule out primary *T. gondii* infections in nearly three quarters of women with positive IgM

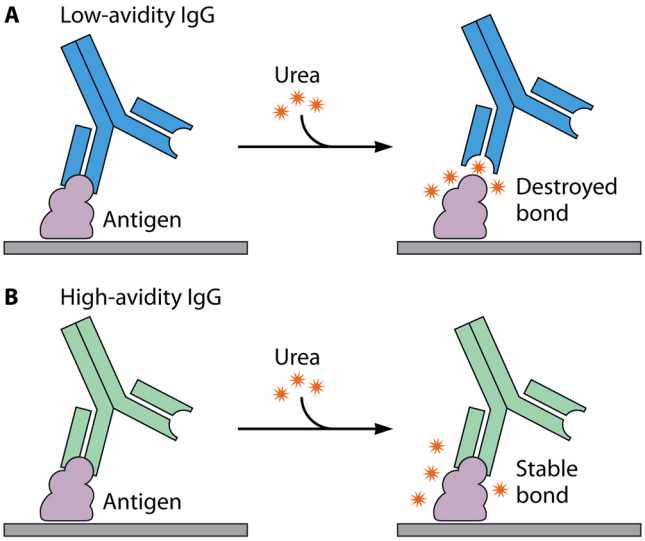


**FIG 2** Principles of low and high IgG avidity. Antigen-bound low-avidity IgG dissociates from antigens in the presence of mild protein denaturants such as urea (A), and high-avidity IgG remains bound to antigens (B).

serum tests during their early pregnancy (19, 32). Moreover, a high IgG avidity in pregnant women before the first trimester of pregnancy can indicate later infections, which makes this test useful in the beginning of pregnancy. However, this finding does not exclude the possibility of fetal involvements during the pregnancy (33). Therefore, investigation of a high-avidity titer at the end of pregnancy does not rule out possible acquired infections in the first or second gestational trimester (20). However, studies have shown that high-avidity titers in pregnant woman suggest possible decreases in risk of fetal infections. Probability of congenital transmissions from infections acquired a few weeks before conception is extremely low or even zero (9, 18, 31, 34, 35).

## STUDIES OF *T. GONDII* IGG AVIDITY IN PREGNANCY

The value of IgG avidity, as a useful tool for the discrimination of recent from past *T. gondii* infections, was first described in the 1980s (18, 36). Investigators (37–39) have supported the usefulness of IgG avidity assessment in diagnosis of recent *T. gondii* infections. However, the exact time of infection acquisition has not been estimated by these investigators. Use of avidity in *T. gondii* infections was reported by Lappalainen et al. in 1993, who assessed serodiagnostic methods in prenatal screening of primary *Toxoplasma* infections acquired during pregnancy in Helsinki (38). A total of 44,181 serum samples were collected consecutively from 16,733 pregnant women during each trimester. A sensitive $\mu$-capture (IgM) ELISA was primarily used for all IgG-containing samples, and positive results were reassessed using IgM immunoblotting and indirect IgM ELISA. For the first time, an assay assessing Toxo IgG avidity was used under screening conditions. Results showed that IgG avidity assay was a highly specific and sensitive tool for the verification of acute primary *Toxoplasma* infections in pregnancy (38). Core studies were carried out between 1996 and 2002, explicitly showing the clinical usefulness of Toxo IgG avidity in identification of primary infections in pregnant women. Results from these studies were significantly similar to each other within the regions of which the major results are discussed later.

## MATURATION DATA SHOW THAT TOXO IgG AVIDITY CAN BE USED FOR THE ESTIMATION OF INFECTION TIME

IgG affinity, which is relatively low after primary antigenic challenges, increases within subsequent weeks and months via antigen-driven B-cell selections. This results in increased complementarities of antigen-antibody binding complexes (20). Sensini et al. collected serial serum samples from patients with primary toxoplasmosis in three

Italian hospitals (Perugia, Treviso, and Bologna) and studied the maturation of IgG avidity (14). They reported increases in IgG avidity from 3.5% in the first month to 38.7% in the first year of infection onset and concluded that the IgG avidity assay was a valuable tool for the serodiagnosis of acute *T. gondii* infections and could predict the stage of infection. The majority of pregnant women with primary infections exhibited low avidity for 3 to 4 months following the infection, with AI values shifting to intermediate/moderate ranges for 1 to 2 months prior to reaching high AI levels. Therefore, a high-avidity value during the first trimester is a strong indicator that infection occurred more than 4 to 5 months earlier, before conception. However, a low-avidity value in the third trimester strongly suggests that the infection occurred within the prior 4 months, after conception.

## IgG AVIDITY IS MORE HELPFUL THAN TOXO IgM FOR THE IDENTIFICATION OF PRIMARY *T. GONDII* INFECTIONS

As previously described, Toxo IgM detection includes high sensitivity but low specificity for the identification of primary toxoplasmosis. A possible reason for this poor specificity is that natural IgM molecules sometimes react with *Toxoplasma* antigens in the absence of infection (14, 17). These natural antibodies primarily include IgM (40) and rarely IgG classes (41), which significantly vary in electrophoretic analyses (42). The antibodies are rarely detected in neonates and infants under 6 months of age. They can be present in pregnant women for the entire gestation (43) or for a limited time (44). Another reason includes the long-term persistence of Toxo IgM for several months or years following the primary infection in some individuals (4, 6, 14–16). These data, in addition to the *Toxoplasma* avidity maturation timeline discussed above, indicate that patients with IgM persistence would display an IgM-positive but high *Toxoplasma* avidity test results if tested 5 to 6 months after the infection. Researchers from European countries have reported that high IgG avidity values exclude acquisition of the infection in the last 3 months and indicate low risks of CT acquisitions (20). Liesenfeld et al. reported that 51.9% of 125 serum samples included high-avidity values in IgM-positive pregnant women, essentially ruling out recent infections. They studied serum samples from pregnant women during the first trimester and concluded that IgG avidity testing of women in their first trimester of pregnancy was a valuable confirmatory test for the exclusion of recently acquired *T. gondii* infections, especially in U.S. laboratories, in which usually a single serum sample was available for the assessment (20). Differences in results between the two analyses (IgM serology and avidity test) might be attributed to the presence of IgM, which could persist from months to years after acute *T. gondii* infection in some cases. Presence of the specific Toxo IgM in chronic stages of the infections may lead to misinterpretation of the results and cause serious concerns leading to unnecessary abortions (17, 20). Therefore, presence or absence of IgM is not an absolute indication of recent infection (39, 45). Thus, the avidity test is extremely important, and the infection can be managed if a differential diagnosis of active *Toxoplasma* infection is carried out on time.

In a study of 99 pregnant women with positive IgG against *Toxoplasma* in 2014, high-avidity values of IgG were seen in nearly 80% of the women with positive IgM tests at the beginning of their pregnancy (46). Of 99 samples used for IgG avidity testing, five samples were positive for IgM. None of the samples included low-avidity indices; however, four samples included high-avidity indices and one sample included a borderline IgG avidity index. All of these five cases were in their first trimester of pregnancy. These findings showed actual prolonged titers of IgM. In fact, these women acquired the infection some time ago and, thus, excluded acquisition of primary infection during early pregnancy. The excellent specificity (97.6%) and negative predictive value (95.6%) suggested that IgM-negative samples with high-avidity indices were indicative of chronic infections and, hence, highlighted the critical role of IgG avidity testing as the best method to exclude primary infections. Recently, Abazaj et al. studied 152 pregnant women in their first months of pregnancy (3 to 12 weeks) and assessed the women for anti-*Toxoplasma* IgM, IgG, and IgG avidity values using ELISA.

The researchers concluded that use of IgG avidity was a significant approach in diagnosis of acute infections, especially in the case of pregnant women (47). Similar results have been reported in other studies (48, 49) in which high proportions of IgM-positive samples with high IgG avidity antibodies were found in the first trimester of pregnancy. Therefore, pregnant women with IgM-positive and high IgG avidity results may have nonrecent primary *Toxoplasma* infections in IgM persistence settings. In any case, high IgG avidity results suggest a low risk of vertical *Toxoplasma* transmission, provided that the testing is performed in the first trimester of gestation. The IgM testing alone incorrectly classifies these women as populations with increased risks of intrauterine *Toxoplasma* transmissions. As shown in Fig. 1, IgM-positive results may highlight two various time lengths from the primary infection whether the patient IgM reversion pattern belongs to the common or persistence pattern (patterns A and B, respectively). If the pattern is A, the IgM test result highlights primary infection within the last 3 months (postconception) and, hence, increased risk of intrauterine transmission of *T. gondii*. If the pattern is B, the IgM test result highlights primary infection within the last 6 months approximately (preconception) and, thus, low risk of intrauterine transmission. However, it is not possible to exactly predict the IgM seroreversion pattern of the patients. Therefore, use of the IgM results to estimate time of the *Toxoplasma* infections is not applicable. In contrast, Toxo IgG avidity testing is an excellent tool for distinguishing between the two possible primary infection onset times. Low-avidity test results highlight infections from the last 3 months and an increased risk of transmissions. In contrast, high-avidity results highlight infection onset before conception with very low risk of transmission.

## IgG AVIDITY TESTS USED IN COMBINATION WITH IgM, IgA, IgG, AND IgE

Research laboratories in Europe and the United States have shown confirmatory positive IgM test results using additional tests in various combinations (38, 39, 50). Diagnostic single-serum assays were carried out for *Toxoplasma*-specific IgM, IgA, IgG, and IgE and various combinations of these antibodies in 20 European reference centers. A panel of 276 serum samples, including 73 serum samples from seroconverted patients within the past 3 months (acute infections), 49 serum samples from seroconverted patients within the past 3 to 12 months (convalescences), and 154 serum samples from patients with two IgG-positive samples during the past 12 months (past infections), were tested using 20 *Toxoplasma* antibody assays and 195 combinations. Overall, assays with high diagnostic sensitivity showed poor diagnostic specificity. No assay alone could reliably differentiate between acute and past infections. In fact, no single or combined assay was able to distinguish convalescence from other *Toxoplasma* infection phases. However, sequential use of highly sensitive IgM assays and methods investigating IgG avidity or stage specificity has achieved excellent diagnostic efficiencies. Indeed, IgA and IgM assays were less appropriate for confirming positivity of *Toxoplasma* IgM (50).

## STUDIES ON *T. GONDII* IgG AVIDITY IN NEWBORNS

Although avidity test results can be achieved in pregnancy, detection of IgG avidity in newborns is still not clarified, and a few avidity studies on newborns have reported low-avidity values in CT-infected neonates (35, 51). The IgG avidity test has been suggested as an effective tool for the diagnosis of acute toxoplasmosis in pregnant women, showing 100% sensitivity and 92.7% specificity (46, 52). However, little is known about the importance of this test in newborns. A study by Buffolano et al. (35) investigated roles of IgG avidity testing in detection of CT in newborns. Data demonstrated that a majority of the infected newborns included low values of avidity, reflecting maternal values. To investigate this association, Fonseca et al. (53) reported that newborns exposed to *T. gondii* with low IgG avidity included higher serum levels of specific IgM and IgG and demonstrated more severe CT symptoms compared to those exposed to *T. gondii* with high IgG avidity. These newborns had a 15-fold greater

risk of developing CT compared to that of newborns exposed to *T. gondii* with high IgG avidity. These data were not previously described in literatures (53).

## STUDIES ON *T. GONDII* IgG AVIDITY AND PCR

Contributions of IgG avidity and PCR to early diagnosis of toxoplasmosis in pregnant women were recently investigated by Berredjem et al. (54). In total, 143 serum samples from pregnant women were assessed. Results included 57 seropositive samples, with 30 (52.6%) IgG positive and IgM negative and 27 (43.8%) IgG positive and IgM positive. In nine samples, IgG avidity was low, suggesting acute infections. Moreover, three samples showed intermediate avidity. *Toxoplasma* DNA was detected in nine low-avidity and zero intermediate-avidity samples using PCR. They concluded that the IgG avidity test was a useful assay for serum samples from pregnant women with positive Toxo IgM. A negative PCR result in combination with positive IgG/IgM suggests past infection, which is excellent for serological samples with uncertain or doubtful results, especially for samples with intermediate avidity. The most surprising results of this study included the high titers of *Toxoplasma* antibodies, low values of avidity, and presence of the parasite DNA and were associated with the existence of acute toxoplasmosis. Such a study is important because this type of study avoids uncomfortable procedures, including multiple time-consuming and costly tests as well as subsequent unnecessary medical administrations (54).

A prospective study investigated Toxo IgG avidity in 146 pregnant women who were positive for *T. gondii* IgM. Multiplex nested PCR was carried out for DNA of *T. gondii* from amniotic fluid, maternal blood, and umbilical cord blood. The multiplex nested PCR on DNA from amniotic fluid or materials at birth was positive in nine women with low IgG avidity values. Of these nine women, three women presented CT. None of the pregnant women with high or threshold avidity values presented positive PCR results in their amniotic fluid. No diagnoses of CT were available in women with negative PCR of their amniotic fluid samples. Therefore, the authors concluded that use of IgG avidity in amniotic fluids in combination with PCR was necessary for the diagnosis of CT (34). Although the avidity test is claimed to be highly specific and sensitive in detection of recent infections, the avidity test is potentially misleading if used alone in serum samples with low or borderline-avidity antibodies with negative or positive IgM titers, respectively (47). Similar conclusions have previously been published by Iqbal and Khalid, who reported negative PCR results for IgM-negative samples with low-avidity antibodies. Furthermore, they reported that two samples with borderline avidity and positive IgM were negative for *T. gondii* DNA (49).

## COMMERCIALLY AVAILABLE TOXO IgG AVIDITY ASSAYS

Table 1 lists the currently available Toxo IgG avidity assays. The list includes five ELISAs, an immunoblot assay, and six automated fluorescence- or chemiluminescence-based assays. In total, 11 of these 12 assays use a dissociating buffer, while one (Abbott Architect, USA) uses a proprietary *Toxoplasma* antigen reagent to inhibit binding of high-avidity IgG to *Toxoplasma* antigens covalently linked to the solid phase. The Liaison diagnostic system (DiaSorin, Saluggia, Italy) is the first fully automated immunoassay that is based on chemiluminescence and antigens bound to magnetic microparticles. It is the first assay to allow assessment of the *Toxoplasma*-specific IgG avidity index at low levels of the specific IgG. The avidity index allows specimen classification as low (avidity index of $<0.2$), moderate (avidity index of 0.02 to 0.25), and high (avidity index of $>0.25$) avidities. The Liaison system is a successful system for excluding recently acquired *T. gondii* infections ($<4$ month) in pregnant women and substantially decreases the necessity of follow-up tests (55). The Vidas system uses an automated enzyme-linked fluorescent assay, which enables quantitative assessment of the specific IgG against *T. gondii*. In 1998, the Pelloux group evaluated solid-phase receptacles coated with *Toxoplasma* membrane and cytoplasmic antigens, including a disposable tip device (56). High-avidity indices allow exclusion of recent infections ($<4$ months), whereas low-avidity indices highlight possible recent infections. However, the later

**TABLE 1** Commercially available *Toxoplasma gondii* IgG avidity kits

| Manufacturer (test name) | Method[a] | Dissociating agent | Low-avidity score[b] | Borderline-avidity score[b] | High-avidity score[b] | Interpretation for high avidity |
|---|---|---|---|---|---|---|
| Ani Labsystems | EIA | Urea | <15 | 15–30 | >30 | Excludes infections in the last 3 mo |
| Abbott (Architect) | CMIA | None[c] | <50 | 50–60 | >60 | Excludes infections in the last 4 mo |
| Diesse | ELISA | Urea | <30 | 30–40 | >40 | Excludes infections in the last 3 mo |
| Euroimmun | ELISA | Urea | <40 | 40–60 | >60 | Unspecified |
| DiaSorin (Liaison) | CLIA | Urea | <20 | 20–25 | >25 | Excludes infections in the last 4 mo |
| Mikrogen | IBL | Urea | N/A | N/A | N/A | Excludes infections in the last 2 to 6 mo according to the antigens |
| Bio-Rad (Platelia) | ELISA | Urea | <40 | 40–50 | >50 | Excludes past infections of over 20 wks but does not exclude with certitude more recent infections |
| Roche (Elecsys) | V-CIA | Guanidine chloride | <70 | 70–79 | ≥80 | Excludes infections in the last 4 mo |
| SFRI Laboratoire | ELISA | Urea | <25 | 25–35 | >35 | Excludes infections in the last 3 mo |
| TestLine | EIA | Urea | <30 | 30–35 | >35 | Excludes infections in the last 4 mo |
| bioMérieux (Vidas) | ELFA | Urea | <20 | 20–30 | >30 | Excludes infections in the last 4 mo |
| Virion/Serion | ELISA | Urea | <45 | 45–50 | >50 | Excludes infections in the last 3 to 4 mo |

[a]EIA, enzyme immunoassay; CMIA, chemiluminescent microparticle immunoassay; ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescent immunoassay; IBL, immunoblotting; V-CIA, voltage-induced chemiluminescent assay; ELFA, enzyme-linked fluorescence assay; N/A, not applicable.
[b]Avidity index values are formatted as percentages for consistency.
[c]This assay uses *Toxoplasma gondii* antigens that block attachments of high-avidity IgG to *Toxoplasma gondii*-coated microparticles.

indices cannot exclude older infections. This finding was verified by other studies using a similar assay on other serum samples (57). A comparison between Vidas and Labsystems IgG avidity index assays has shown an overall correlation coefficient for both test results as 0.80 (58). The Abbott Architect assay uses no dissociating agents to release low-avidity IgG from the solid phase. In this method, binding of high-avidity antibodies to the solid phase is suppressed using proprietary *Toxoplasma* antigen reagents. This alternative assay was developed by the manufacturer to avoid possible detrimental effects of dissociating agents on the Architect complex fluidic systems (59). Gay-Andrieu et al. evaluated this assay and reported a correlation value of 0.87 between the Architect and Vidas avidity assays (60).

The Elecsys Toxo IgG and IgM assays (Roche Diagnostics GmbH, Mannheim, Germany) have been validated for immune status screening and monitoring in pregnant women (61, 62). A novel assay, the Elecsys Toxo IgG avidity assay, has recently been developed. This assay is based on the Toxo IgG assay, which is an *in vitro* diagnostic assay to assess the qualitative avidity of IgG against *T. gondii* in human plasma samples and serum samples. In 2012, Murat et al. (63) used two serum sample sets ($n = 291$ and $n = 255$) to compare the Elecsys assay with the Vidas (bioMérieux, France) and Architect (Abbott, USA) assays. The general agreement rates included 74% between the assays of Elecsys and Vidas and 83% between the assays of Elecsys and Architect. None of these assays detected high-avidity antibodies in serum samples collected at up to 4 months of infection. Avidity values of 90% or greater were reported exclusively in serum samples collected after 9 months of infection using Elecsys and Architect assays. Almost all avidities of less than 19% using the Elecsys assay and less than 17% using the Architect assay were linked to serum samples collected at less than 3 and 2 months of infection, respectively (63). The immunoblot assay, fairly novel to the market, uses urea as a dissociating agent and needs a scanner to assess the band strength. Low avidity requires at least 50% decreases in band intensity when urea-treated blot strips are compared to untreated ones. Although most primary and past *T. gondii* infections were correctly identified in immunoblot assay, it showed no significant performances or achievements other than those of the conventional Toxo IgG avidity assays.

Villard et al. analyzed four assays, Architect Toxo IgG avidity (Abbott, USA), Vidas Toxo IgG avidity (bioMérieux, France), Platelia Toxo IgG avidity (Bio-Rad, USA), and Liaison Toxo IgG avidity II (DiaSorin, Italy) as the most commonly used assays in French biology laboratories and reference laboratories abroad (64). These fully automated assays have been designed on the basis of excluding acute infections while including professional results. The Architect assay, which used recombinant antigens, provided

the best results to detect latent infections in the presence of persistent IgM. This suggested that the recombinant antigens might later be used in toxoplasmosis assays, and the antigen types used in antibody recognition were crucial. For example, IgG against antigens previously identified (e.g., GRA7, GRA8, and ROP1) significantly matured earlier compared to those against antigens later identified (e.g., SAG1 and MAG1) (65). This study has shown that the avidity test can identify latent *Toxoplasma* infections in pregnant women who show specific IgG and IgM against *Toxoplasma* in primary pregnancy tests. However, the assay includes certain disadvantages because there are no definitive findings when assessing other immune-compromised patients treated for toxoplasmosis. In these special cases, several tests, such as serological, culture-based, and molecular (PCR) tests, must be carried out in combination for optimal diagnoses (65). In a study by Genco et al. (66), performances from the Liaison XL system of IgG and IgM immunoassays for the diagnosis of *T. gondii*, cytomegalovirus (CMV), and rubella virus infections were compared with the performance of the Architect system. Findings showed that the overall agreements between the Liaison XL and Architect assays included 99 and 92% for Toxo IgG and IgM, respectively, and concluded that all assays were appropriate to test patients with suspected primary *T. gondii*, CMV, and rubella virus infections (66).

## ISSUES AND CONTROVERSIES

**How should the intermediate/moderate Toxo IgG avidity results be used?** The major use of the Toxo IgG avidity test is based on low or high avidity. Low avidity suggests an increased risk of intrauterine transmission, whereas high avidity suggests a low risk of intrauterine transmission in the first trimester (6, 7, 9, 18, 31, 32, 34, 35). Intermediate or moderate IgG avidity findings have been considered difficult to interpret for risk assessment purposes (54). Nowadays, researchers believe that intermediate/moderate Toxo IgG avidity must be interpreted with caution, and additional studies with other commercial Toxo IgG avidity and molecular assays are needed to better understand the significance of IgG moderate/intermediate/gray-zone avidity for verification of the diagnosis of primary *T. gondii* infections in pregnancy. In 2017, Berredjem et al. (54) reported that IgG avidity alone did not show acute or chronic infection status in three women with intermediate IgG avidity, whereas PCR showed no results with both gene targets reporting chronic infections in these women. However, DNA of *Toxoplasma* was present in nine samples with low avidity using PCR, demonstrating acute infections.

**Should Toxo IgG avidity be assessed only for the samples with positive *Toxoplasma gondii* IgM?** Many laboratories using Toxo IgG avidity to discriminate recent from nonrecent *T. gondii* infections follow a reflexive algorithm by which only Toxo IgG-positive samples that are also Toxo IgM positive are tested for Toxo IgG avidity (67, 68). However, IgM may be temporary or absent and demonstrate low IgG avidity in rare cases, suggesting increased risk of intrauterine transmission (69). Therefore, the reflexive algorithm that depends on IgG avidity testing of IgM-positive samples may miss a few primary infections. Researchers have recommended that serum samples are first tested for Toxo IgG and IgM and then IgG-positive samples are tested for Toxo IgG avidity regardless of the IgM results (67). In addition to identifying IgM-negative patients with low IgG avidity, this approach detects the small number of patients with IgG-negative and IgM-positive results, indicative of very recent *T. gondii* infection. However, seroconversions must be documented to ensure that IgM results are really positive. Studies have shown that a combination of assessments for IgG avidity antibodies with sensitive tests for *Toxoplasma*-specific IgM includes the highest predictive value based on the infection time (55, 67, 68).

**Should all pregnant women be screened for evidence of primary *Toxoplasma gondii* infection?** In several countries (except France and Austria), systemic screening of all pregnant women for Toxo antibodies is not routinely carried out due to multiple factors, such as costs, demographic characteristics, availability of appropriate tests, and relatively low occurrences of acute infections (70). If Toxo IgG and IgM are detected during the first 2 months of gestation, results are quite promising. (i) Since IgG and IgM

are absent in blood serum at this early gestational age, the possibility of recent infection is excluded within the past 7 days in the absence of recent contaminations of less than 1 month. However, serological follow-ups are necessary for these cases, depending on the clinical situation. In these cases, people are considered susceptible to infection, and hygienic and dietary preventive measures must be provided for pregnant women and immunocompromised patients. (ii) Presence of specific IgG and absence of IgM at this early gestational age almost always demonstrate classic serological patterns of past infection, and hence serological follow-ups can be discontinued since immunity is assumed to protect the fetus from reinfection. Nonetheless, use of a second serology test after 3 weeks is usually recommended to monitor possible increases in IgG levels. Stable rates of IgG demonstrate chronic toxoplasmosis. Based on the methods, significant increases in IgG contribute to assessing IgG avidity. Reinfections or reactivations are highly suspected in cases with high IgG avidity. In contrast, the contamination date cannot be specified if IgG avidity is low or equivocal in pregnant women; thus, adapted management must be initiated depending on the gestational age. Serological follow-up is unnecessary when an immunocompetent subject presents this situation. (iii) Absence of specific IgG and presence of IgM suggest very recent *T. gondii* infection and an increased risk of vertical transmission. Patients must be followed up to document seroconversion and tested for Toxo IgG avidity. High or intermediate avidity suggests a low risk of congenital infections, while low avidity suggests an increased risk of congenital infections (37, 71). If results suggest low avidity, patients must be informed that vertical transmission is not concluded inevitably and additional techniques must be used to detect fetal infection. (iv) Presence of specific IgG and IgM demonstrates recent primary infection and suggests similar consequences to IgG-negative and IgM-positive situations (67).

## CONCLUSION

The avidity tests are particularly valuable when no screening programs are available, and a single serum sample from anti-*Toxoplasma* IgM-positive pregnant women in the first trimester of pregnancy is available for serological diagnosis. Primary findings from IgG avidity tests suggested that low avidity in puerperae generally showed recently acquired infections, which increased risks of intrauterine transmissions to fetuses/newborns (31, 34, 38). However, studies have shown that IgG avidity can persist for several months after a recent infection (14, 55, 72, 73). It can be a normal reaction in some people after infections because of immunological changes during pregnancy or responses to antibiotics (14, 72). In contrast, a common consensus is that the IgG avidity test is best used to rule out recently acquired infections. Depending on the methods, presence of high-avidity IgG can rule out the occurrence of acute infections within the past 3 to 4 months and demonstrates low risk of intrauterine transmission. Therefore, the assessment includes the greatest value if carried out in the first trimester of gestation. High-avidity results from the late second or third trimester cannot be interpreted, as the infection was not acquired within the first 3 to 4 months of gestation. Late use of the test may lead to unnecessary diagnostic amniocentesis, treatment of the mother, and concerns for the partner (74). Moreover, it increases the possibility of unnecessary abortions. Thus, optimal timing for the first antenatal *Toxoplasma* serology test is the initiation of pregnancy. In some patients, it is possible to enhance the final clinical decision by collecting follow-up samples after 3 to 4 weeks and before amniocentesis. If IgG avidity is low and stable in the first trimester, chances of infection occurrence in pregnancy are low and risk of fetal infection is even lower. However, if the avidity increases dramatically, women must be diagnosed via prenatal amniocentesis. Although IgG avidity tests include a limited potential to assess the initiation of primary infection, high IgG avidity certainly rules out infection in pregnant women with persistent Toxo IgM positivity within the first 4 months of pregnancy. However, intermediate/moderate IgG avidity results are difficult to interpret for risk assessment (54). Intermediate/moderate Toxo IgG avidity must be interpreted with caution, and additional studies with other commercial Toxo IgG avidity and molecular assays must be carried out to better understand the significance of IgG moderate/intermediate/gray-

zone avidity in verified diagnosis of primary *T. gondii* infection in pregnancy. A vast majority of patients with primary *T. gondii* infection demonstrate both low *T. gondii* IgG avidity and detectable *T. gondii* IgM; however, rarely is only one of these abnormal results present. Therefore, maximum detection of primary *T. gondii* infection, particularly when the first sample is collected within the first trimester, requires that both *T. gondii* IgG avidity and IgM testing are carried out on *T. gondii* IgG-positive samples (67, 68, 75).

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